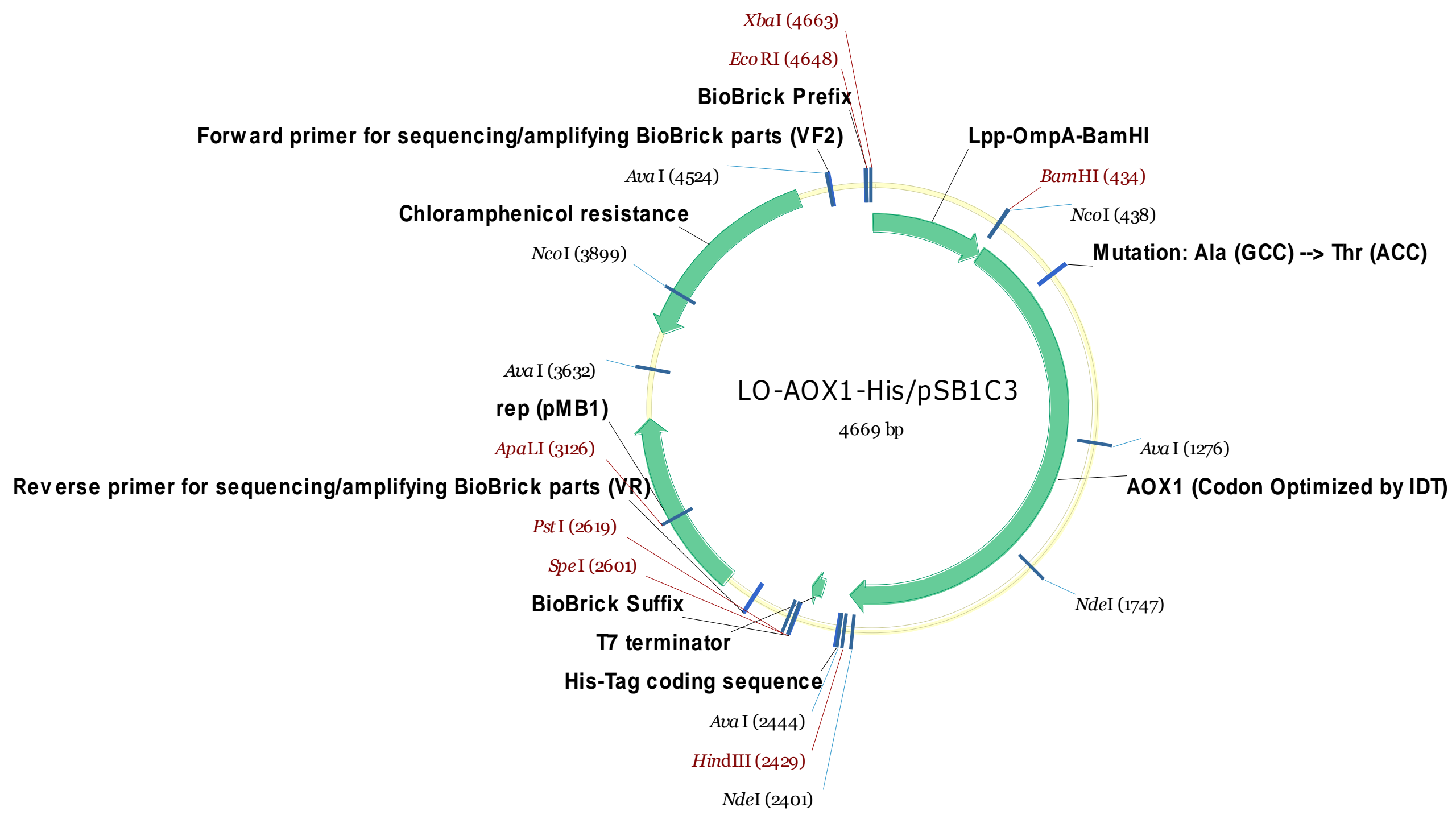


**LO-AOX1-His/pSB1C3**



# Strategy

- Vector: LO-BamHI/pSB1C3 (2509 bp) → BamHI + PstI
- Insert: AOX1-His amplified from AOX1/pET-29b (2193 bp) → BamHI + PstI

# Primer Design

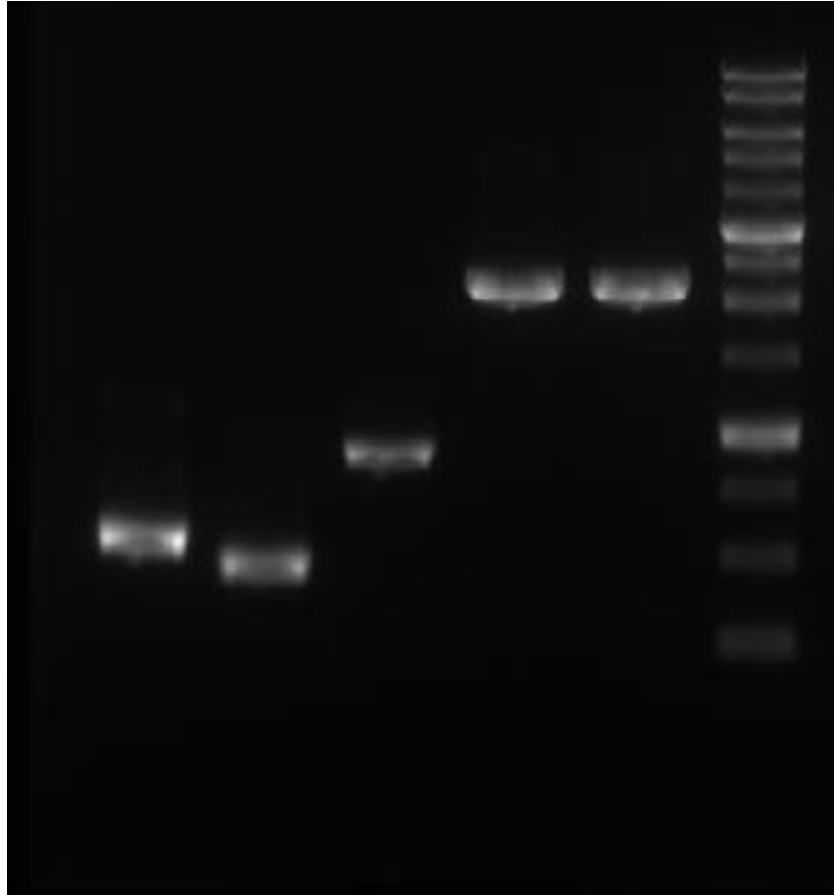
- AOX1-BamHI-F:

- 5'- GAACGAGGATCCATGGCTATTCCGGAAGAGTT -3'

- T7T-SpeI-PstI-R:

- 5'-  
AAGAGACTGCAGCGGCCGCTACTAGTATATAGTTCCTCCTTTCAG  
-3'

# PCR with KOD-plus



- Date: 2016.08.17

- Lane:

1. Pcons-RBS-LO-BamHI

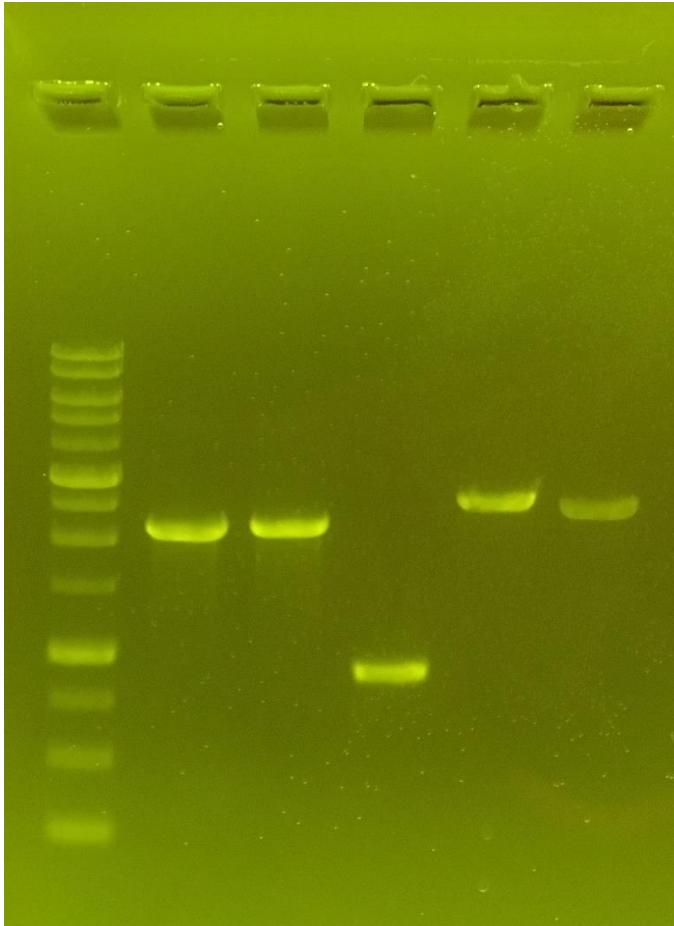
2. LO-BamHI

3. GFP

4. **AOX1**

5. AOX2

# Ligation



- Date: 2016.08.31

- Lane:

1. AOX1: BamHI + PstI

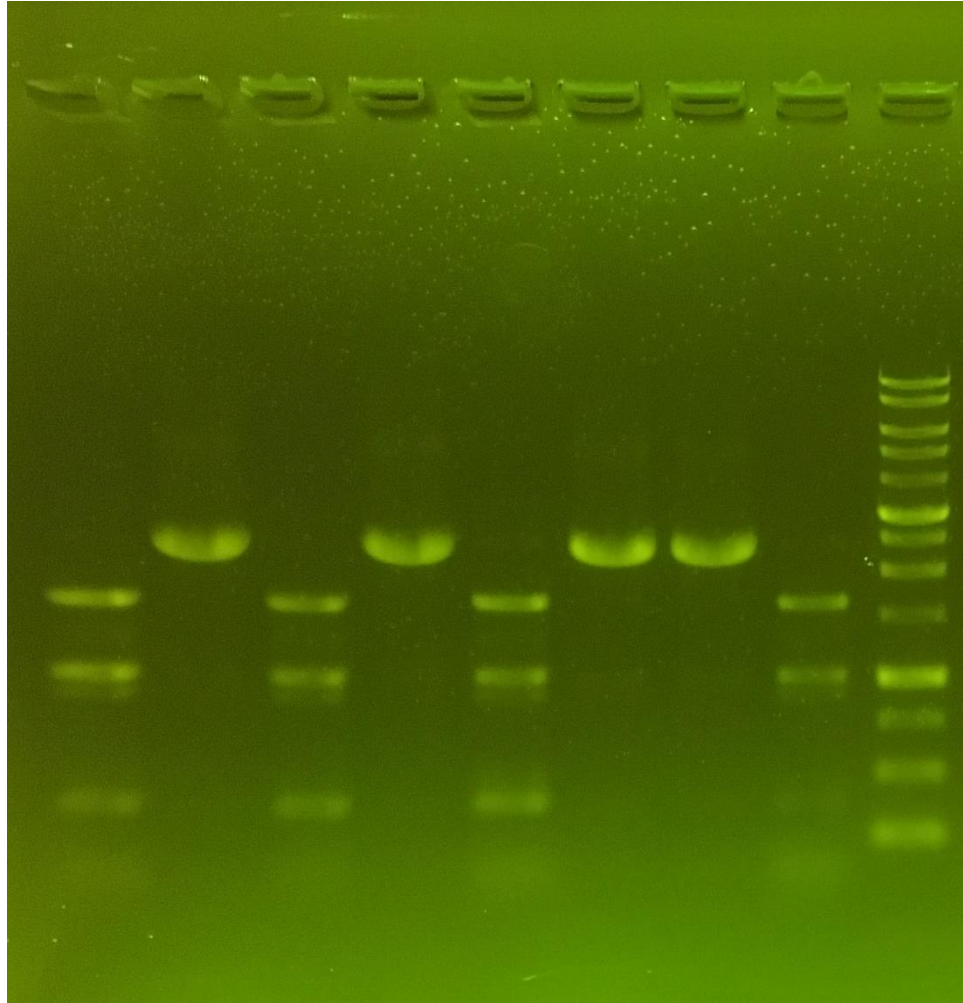
2. AOX2: BamHI + PstI

3. GFP: BamHI + PstI

4. Pcons-RBS-LO-BamHI/pSB1C3  
#P6: BamHI + PstI

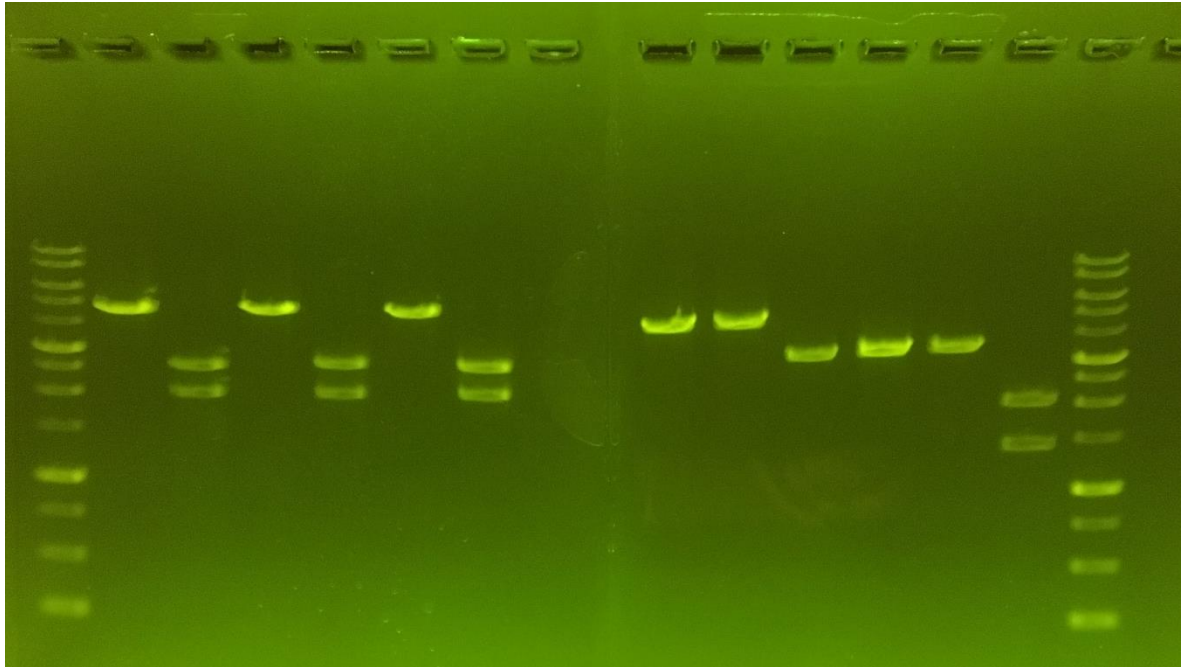
5. LO-BamHI/pSB1C3 #L2: BamHI  
+ PstI

# Colony PCR



- Date: 2016.09.02
- Clone: LO-AOX1/pSB1C3
- Primer:
  1. Lpp-XbaI-EcoRI-F
  2. AOX1-PstI-R
- Lane: #L1-1 ~ #L1-8
- #L1-2 → Plasmid extraction  
→ RE check

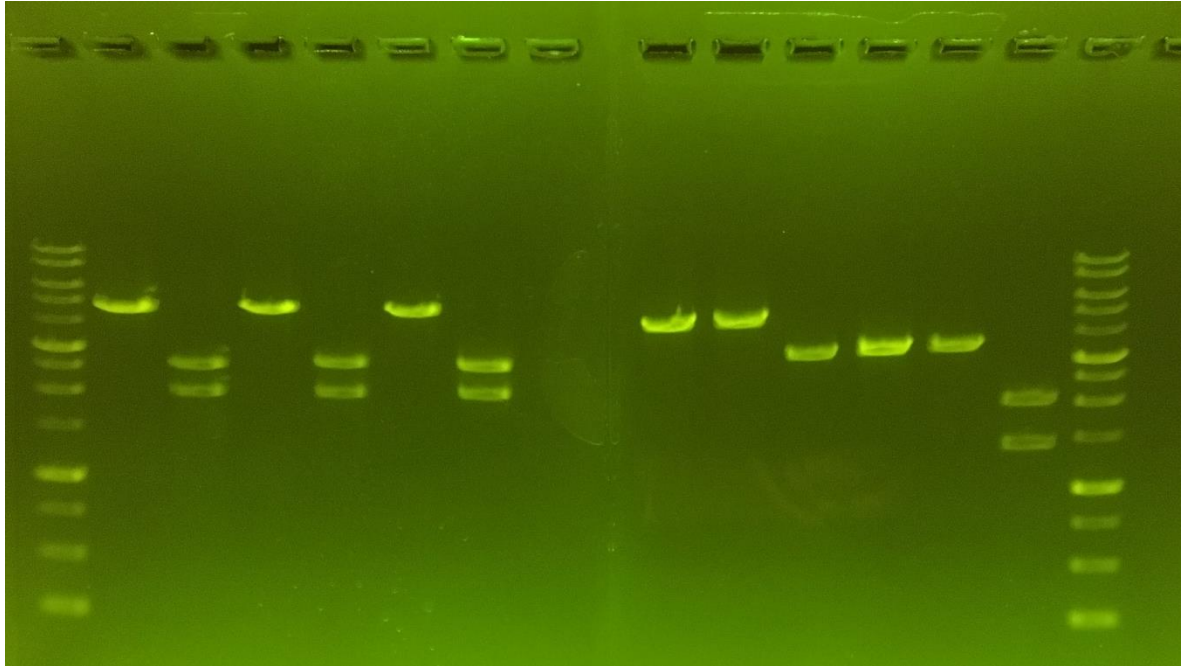
# RE check



- Date: 2016.09.07
- Clone: LO-AOX1-His/pSB1C3
- Lane:
  1. #L1-2: EcoRI
  2. #L1-2: EcoRI + PstI
  3. #P1-5: EcoRI
  4. #P1-5: EcoRI + PstI
  5. #L2-5: EcoRI
  6. #L2-5 : EcoRI + PstI
  7. #P2-2: EcoRI
  8. #P2-2: EcoRI + PstI
  9. #LG-3: EcoRI
  10. #LG-3: EcoRI + PstI
  11. #PG-4: EcoRI
  12. #PG-4: EcoRI + PstI



# RE check



- Date: 2016.09.07
- Clone: LO-AOX1-His/pSB1C3
- Lane:
  1. #L1-2: EcoRI
  2. #L1-2: EcoRI + PstI
  3. #P1-5: EcoRI
  4. #P1-5: EcoRI + PstI
  5. #L2-5: EcoRI
  6. #L2-5 : EcoRI + PstI
  7. #P2-2: EcoRI
  8. #P2-2: EcoRI + PstI
  9. #LG-3: EcoRI
  10. #LG-3: EcoRI + PstI
  11. #PG-4: EcoRI
  12. #PG-4: EcoRI + PstI

# Sequencing

- Date: 2016.09.14
- Sequencing primers: VF2, VR
- Clone: LO-AOX1-His/pSB1C3 #L1-2
- File: “2016.09.14\_LO-AOX1-His\_pSB1C3 (#L1-2)”
- Result: correct → make a stock